Fucosyl gangliosides of PC12 pheochromocytoma cells

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Three monosialogangliosides are highly labeled when PC12 pheochromocytoma cells are cultured in the presence of L-[^{3}H]fucose, and two additional monosialogangliosides are labeled to a lesser extent. In contrast, neither of the two disialogangliosides of PC12 cells contains fucose residues. Removal of sialic acid and fucose by formic acid hydrolysis demonstrated the presence of 3 major 'core' structures in the monosialogangliosides, and a single asialo derivative of the disialogangliosides which has the same chromatographic mobility as one of the monosialoganglioside hydrolysis products. None of the major formic acid hydrolysis products of the PC12 cell gangliosides corresponds to asialo-GM_1, supporting our previous conclusion that PC12 cells do not contain significant amounts of brain-type gangliosides.

Ganglioside *PC12 pheochromocytoma cell* *Glycolipid* *Fucose*

1. INTRODUCTION

We have described the occurrence in PC12 pheochromocytoma cells of 3 major monosialogangliosides and two disialogangliosides, all of which differ in chromatographic mobility from those present in brain [1]. These gangliosides account for approx. 3% of the glucosamine-labeled complex carbohydrates present in the cells, where they occur together with labeled glycoproteins (93%), chondroitin sulfate (1.3%) and heparan sulfate (3.4%). In response to nerve growth factor, PC12 cells extend long processes and acquire other properties similar to those of sympathetic neurons [2]. Treatment with nerve growth factor also produces an almost 3-fold increase in the concentration of labeled gangliosides (dpm/mg cell protein), although no differential effects were seen in the relative labeling of individual ganglioside species after fractionation by thin-layer chromatography (TLC) [1]. Here we have examined the structural relationships between the different PC12 cell gangliosides, and have demonstrated that all of the monosialyl gangliosides contain fucose residues.

2. EXPERIMENTAL

The labeling of PC12 cells and rat brain with [6-^{3}H]glucosamine or L-[1-^{3}H]fucose, extraction of gangliosides, and their purification by ion exchange and silicic acid column chromatography were performed as in [1]. Mono- and disialogangliosides were further fractionated by chromatography on DEAE–Sepharose CL-6B [3] and desalted by gel filtration on Sephadex LH-20 [1]. Enzyme treatments were carried out as described and the glycolipid products were isolated by silicic acid chromatography [1]. For fucosidase treatment, gangliosides were incubated with 0.5 units bovine epididymis α-L-fucosidase or 0.25 units bovine kidney α-L-fucosidase (both from Sigma), with a second addition of the same amount of enzyme after 8 h. Fucosidase digestions were carried out for a total of 24 h at 37°C in 50 mM sodium citrate buffer (pH 6.0) containing 1 mg sodium deoxytaurocholate/ml.

Asialo-GM_1 (prepared from labeled brain gangliosides) and desialylated/defucosylated PC12 cell gangliosides were obtained by treatment with 0.1 N

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formic acid for 2 h at 100°C [4]. Formic acid was removed by lyophilization, and after partitioning in a biphasic system of chloroform/methanol/water (8:4:3, v/v) followed by centrifugation, the upper phase was removed and the lower phase washed 3 times with a 'theoretical upper phase' consisting of chloroform/methanol/water (3:48:47, v/v) [5]. The lower phase was then evaporated to dryness and redissolved in a small volume of chloroform/methanol for TLC. Ganglioside fractions and the products obtained by enzyme or formic acid treatment were chromatographed on silica gel 60 HPTLC plates (Merck) developed with chloroform/methanol/0.2% aqueous $CaCl_2$ (50:40:10, v/v), and labeled bands were detected by fluorography as in [1]. Globoside was generously provided by Dr Robert Ledeen. The radiochemical purity of the L-[^{3}H]fucose used for labeling PC12 cells, and the identification of the labeled sugar present in the purified gangliosides (after release by hydrolysis for 2 h in 1 N formic acid at 100°C) were established by TLC on MN 300 cellulose Uniplates (Analtech) using 1-butanol/ethanol/water (25:16:9, v/v), and 1-butanol/pyridine/water (6:4:3, v/v).

3. RESULTS AND DISCUSSION

Gangliosides were isolated from PC12 cells labeled with L-[^{3}H]fucose or [^{3}H]glucosamine, and further fractionated into mono- and disialo species by chromatography on DEAE–Sepharose. TLC demonstrated that all of the monosialogangliosides were labeled by fucose (fig.1) whereas no fucose labeling was seen in the disialogangliosides eluted from the DEAE–Sepharose column. These studies also demonstrated the presence in PC12 cells of a minor monosialoganglioside of low chromatographic mobility (fig.1, arrowhead), which migrates immediately ahead of the disialogangliosides. This monosialoganglioside was previously not resolved from the most rapidly migrating disialoganglioside (which appeared as a broad band near the area where G_{T1b} is found in chromatograms of brain gangliosides [1], fig.1), and is the only monosialoganglioside affected by neuroaminidase treatment.

The faintly labeled ganglioside bands which migrate between the major mono- and disialogangliosides (fig.1, right lane), in the area usually occupied by disialogangliosides of brain ([1] fig.1), were found to be eluted from DEAE–Sepharose (with 0.02 M potassium acetate) as a broad peak after the major fraction of monosialogangliosides, but before the disialogangliosides (which require 0.1 M potassium acetate for elution) [3]. These glycolipid bands are not labeled with L-[^{3}H]fucose, and conversely, a minor fucose-labeled ganglioside (fig.1, center lane) was not significantly labeled by [^{3}H]glucosamine. After hydrolysis of the fucose-labeled gangliosides, all of the incorporated radioactivity was identified as fucose by TLC followed

Fig.1. Fluorograph of thin-layer chromatogram of PC12 cell monosialogangliosides labeled with [^{3}H]glucosamine (left), and of the unfractionated gangliosides labeled with L-[^{3}H]fucose (center) and [^{3}H]glucosamine (right). A faint band representing the minor monosialoganglioside having a low chromatographic mobility (barely visible in the left lane) is indicated by the arrowhead, below which are the two disialogangliosides.

by fluorography and comparison with a fucose standard.

When L-[^{3}H]fucose-labeled PC12 cells gangliosides were treated with formic acid under conditions reported to desialylate brain gangliosides completely but to produce little change in the remainder of the oligosaccharide [4], gel filtration on Sephadex G-25 demonstrated that essentially all of the fucose is also removed. This finding was not surprising in view of the known acid lability of fucose residues. Since we previously demonstrated that treatment with *Vibrio cholerae* neuraminidase had no effect on the major monosialogangliosides [1], PC12 cell gangliosides were treated with formic acid to determine the number of different oligosaccharide 'core' structures present after removal of both fucose and sialic acid. Formic acid hydrolysis converted the 4 monosialogangliosides into an equal number of new bands (one of which is relatively faint), whereas the two disialogangliosides yielded a single component which migrated on TLC at the same position as the major product obtained from the monosialogangliosides (fig.2). Since a single (but less rapidly migrating) product was also obtained after partial desialylation of the disialogangliosides by neuraminidase [1], the results of formic acid treatment support the assumption that the two disialogangliosides differ from each other only in the location of one neuraminidase-labile sialic acid residue.

Treatment of L-[^{3}H]fucose-labeled PC12 cell gangliosides with bovine epididymis α-L-fucosidase removed approx. 60% of the fucose label in the presence of sodium deoxytaurocholate, whereas bovine kidney α-L-fucosidase had no effect in either the presence or absence of detergent. Only the most rapidly migrating monosialoganglioside band was affected by fucosidase treatment, and the detergent requirement for fucosidase activity indicates that the hydrolyzed fucose residue is not linked to the terminal sugar in the oligosaccharide.

None of the major formic acid hydrolysis products of the PC12 cell gangliosides correspond in chromatography mobility to asialo-G_{M1} obtained by similar treatment of brain gangliosides (fig.3), confirming our previous conclusion that PC12 cells do not contain significant amounts of brain-type gangliosides [1]. Since galactosamine was the only amino sugar detected after complete acid hydrolysis of the PC12 cell glycolipids [1], these

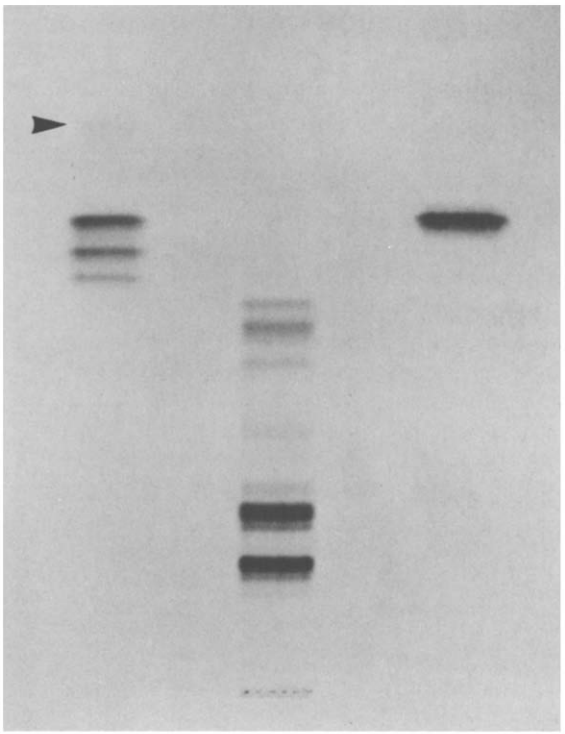

Fig.2. Fluorograph of thin-layer chromatogram showing products obtained after formic acid treatment of [^{3}H]glucosamine-labeled monosialogangliosides (left) and disialogangliosides (right), together with the unfractionated gangliosides before formic acid treatment (center). The position of a minor product resulting from formic acid treatment of the monosialogangliosides is indicated by the arrowhead.

apparently belong to the ganglio series, or are examples of novel gangliosides occurring in the globo or isoglobo series [6–8]. However, they are not simple sialosyl- or fucosylglobosides, since all of the major formic acid hydrolysis products of the PC12 cell glycolipids chromatographed behind a globoside standard. We are currently examining these possibilities in the course of a more detailed characterization of the major PC12 cell gangliosides employing larger amounts of material.

It is noteworthy that the ganglioside composition of PC12 cells is markedly different from that of either adrenal medulla [9–12] or brain. These differences might be due to their tumor origin and properties, but they may also reflect the presence of glycolipids which are abundant during early stages of development but decline in concentration to relatively low levels in mature brain. A minor

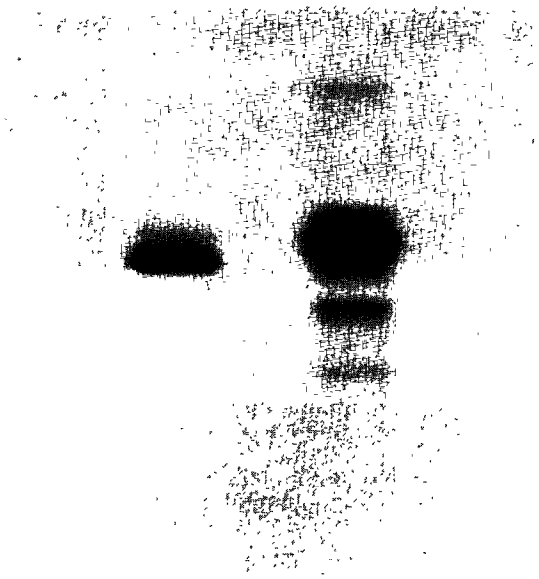

Fig.3. Fluorograph of thin-layer chromatogram comparing the mobilities of asialo-GM_1 (left) prepared by formic acid treatment of [^{3}H]glucosamine-labeled rat brain gangliosides, with the formic acid hydrolysis products of unfractionated PC12 cell gangliosides labeled with [^{3}H]glucosamine (right).

formic acid hydrolysis product of adult rat brain gangliosides (which migrates immeditely ahead of asialo-GM_1) corresponds in chromatographic mobility to the major core structure of PC12 cell gangliosides (fig.3), but its relative intensity was found to be only slightly increased, if at all, in chromatograms of similarly treated gangliosides isolated from [^{3}H]glucosamine-labeled 1-day-old rat brains. However, the possibility remains that this formic acid hydrolysis product of brain gangliosides is derived from glycolipids whose concentration is relatively high only in embryonic brain, and that it is identical to the major core structure present in the PC12 cell gangliosides.

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